GLYCOGEN METABOLISM IN INSECTS: A REVIEW.

By Morris Rockstein, Pullman, Washington.

I. Introduction

Since the early discoveries of Claude Bernard of the importance of glycogen as the storage form of carbohydrates in animals, a wealth of complicated chemical and physiological data have been accumulated concerning its metabolism. Especially rapid advances during the past fifteen years in the field of enzymology have greatly clarified the complex intermediary metabolism of carbohydrates and of glycogen, in many vertebrate and invertebrate animals. Surprisingly, however, of all forms studied, insects, the most numerous of animals, have been least explored in this respect. With the above in mind, this review has been written to include a comprehensive picture of the present knowledge of glycogen metabolism in insects, with the chief aim of pointing to possibilities for further study in this field.

II. Glycogen in Insects

The ability of insects to utilize glycogen has been studied in two different ways; by feeding experiments in which the diet included glycogen and by tests for the presence of the enzyme glycogenase, the latter assuming that glycogen in the diet is normally used. Bertholf (1927) found that honeybees could not utilize glycogen. Fraenkel (1936) showed that the blowfly (*Calliphora erythrocephala*) could substitute many sugars for cane sugar in its diet, including glycogen. This was taken as evidence of the presence of glycogenase in the alimentary canal of this species. In an extensive study in the southern armyworm (*Prodenia eridania*) Babers and Woke (1937) demonstrated the presence of glycogenase in the *tissue* of the midgut alone, although it was present in the lumen of the various divisions of the alimentary canal. They assumed that glycogenase was secreted only by the midgut and suggested its presence elsewhere was the result of elaboration by microfloral inhabitants. Simmons (1939) also demonstrated the presence of glycogenase in the cattle grub (*Hypoderma lineatum*) alimentary canal and that it was secreted by the lining of the mid-intestine itself. Parkin (1940) in a study of the carbohydrases of wood-boring beetles, found no evidence of glycogenase although other polysaccharases like cellulase and hemicellulases were demonstrated in several groups of these phytophagous forms.

The first demonstration of glycogen in insects was made by Claude Bernard (1885) who described fly larvae as "veritable sacs of glycogen." The primary seat of glycogen storage in insects, established by histochemical methods, is the fat body, and secondarily the blood cells and midgut epithelial cells. Yeager and Munson (1941) in an extended study on the incidence of glycogen in the tissues of the southern armyworm (*Prodenia eridania*) gave an excellent recapitulation of the work done on the occurrence of glycogen in the tissues of various insects studied prior to that year. These tissues included muscle, oenocytes and even the malpighian tubules. Wigglesworth, in a later study (1942), found glycogen in the ganglia and in muscle connectives of newly-moulted fourth-instar larvae of *Aedes* mosquitoes, as well as in the epithelial cells of the posterior half of the midgut and in the fat body of the thorax. Snodgrass (1925) points out that the storage of glycogen in the fat body of the honeybee larva, despite its inability to use glycogen from the diet directly, suggests that the fat body corresponds to the liver of higher animals as a site of glycogen synthesis and storage. Babers (1941) comes to a similar conclusion regarding the fat body of insects and the liver of vertebrate forms.

III. The Physiology of Glycogen Metabolism in Insects

Claude Bernard's findings included the important fact that glycogen stores decreased gradually with metamorphosis. This was the first of a long series of reports on glycogen occurrence and its relation to metamorphosis and development. Bataillon and Couvreur (1892) and Bataillon (1893) found that the glycogen content of the silkworm reached a maximum at the end of the larval stage, the chrysalis containing twice as much as the larva. During the pupal stage, glycogen content fell slowly to a minimum at the time of emergence of the adult. Reducing sugars like glucose, on the other hand, increased during metamorphosis to a maximum at three to four days before emergence of the adult from the chrysalis. Vaney and Maignon (1905) reported a similar diminution of glycogen in the silkworm from egg to larval emergence. Only 30% of the original glycogen remained at that time. In contrast to the above, Kaneko (1924) and Minoya (1932) reported no glycogen at all at the time of hatching in the silkworm larva. Kaneko also showed that glycogen increased in the larva to a maximum just prior to pupation. This he associated with the continuous feeding during the larval period on the carbohydrate-

rich mulberry leaves. Nelson, Sturtevant and Lineburg (1924) also showed a similar association of glycogen storage and carbohydrate diet in the honeybee larva. Frew (1929) suggested that glucose in the blowfly larvae (probably *Calliphora* sp.) occurring in two peaks, was probably converted from stored fat and protein, since he could not find any glycogen in the larvae or pupae. Snodgrass (op. cit.) quotes Straus as reporting a low glycogen content in larval honeybee until the third day, rising to a peak at the beginning of pupation. Rudolfs (1929) found that glycogen stores in overwintering eggs of the eastern tent caterpillar (*Malacosoma americana*) decreased to a minimum at hatching. In the larva, glycogen content showed two peaks, with a final high at the end of the prepupal stage and a final minimum at adult emergence. In the bee moth (*Galleria mellonella*), Crescitelli and Taylor (1935) found no glycogen in the prepupal and pupal stages. They assumed that reducing sugars, assumed to be glucose, could therefore arise from fats and proteins. This was partly substantiated by Taylor and Steinbach (1931) who reported a low respiratory quotient (R.Q.) during pupal development of the bee moth. Ludwig (1932) found that glucose increased markedly from the late prepupal to early pupal stages of the Japanese beetle, to a maximum at adult emergence; no data on glycogen were presented. Evans (1932) [illegible] cogen in the sheep blowfly, in contrast to Frew (see above), Babers (1941) questioned the validity of the findings of Evans. However, Evans also reported a steady fall in glucose during the pupal period of the sheep blowfly and of the mealworm. Finally, Yeager and Munson (1941) and Munson and Yeager (1944) reported that blood glycogen in the southern armyworm rose steadily during the larval stage, reaching a maximum at pupation, and falling again during metamorphosis. Babers (1941) found a similar disappearance of glycogen at hatching of the egg, reappearance in the larva, rising to a maximum at pupation and falling again during the pupal period. The paper by Babers has an excellent bibliography on carbohydrate metabolism of insects up to (and including) 1939. Needham (1942) summarized the significance of much of the above work in terms of adaptation by holometabolous insects to their type of metamorphosis; i.e., glycogen is the storage form of energy built up during the feeding larval stage and utilized during pupal development in the final transformation to the imago. He pointed out that in this development, the silkworm used as much as 100%, the eastern tent cater-

pillar 94%, the blowfly (*Calliphora vomitoria*) 73% and the honeybee 94% of the original stores of larval glycogen. Related to growth and development is the process of moulting and the production of a new cuticle. Needham (1931) cited Tichomirov as first noting that the chitin in silkworm was formed just at the time when glycogen stores disappeared. Paillot's excellent studies (1937, 1938) on the silkworm lead him to conclude that "glycogen plays a direct part in the building of a new cuticle." He found glycogen to be heavily deposited in the epidermal cells, and in all the epithelial tissue having a "chitinous" lining, just prior to moulting. Furthermore, the normal storage points of glycogen, fat cells, lost their glycogen at the same time. Since the basic structure of chitin is recognizedly a polyglucoside, these findings are especially important in clarifying our understanding of the intermediary metabolism in the laying down of the chitinous portion of the cuticle, wherever present.

Within the past ten years several workers have attempted to correlate insect flight activity with carbohydrate metabolism. Chadwick and Gilmour (1940) first suggested that the limiting factor in flight duration of *Drosophila repleta* was physiological fatiguing in the form of depleted reserve food stuffs. Williams, Barness and Sawyer (1943) continued this study on *D. funebris* and in *Lucilia sericata* and found a progressive decrease in glycogen concentration with extended flight. They also found that greater endurance in flying by younger insects was correlated with the possession of a higher glycogen content than older animals.

Finally, some workers, especially biochemists and general physiologists working with insects, have attempted to establish the chemical basis of insect glycogen metabolism. This has primarily involved chemical analyses for the presence of phosphorous compounds and studies of respiratory quotients. Davis and Slater (1926, 1928) and Slater (1927), in a study on the aerobic and anaerobic metabolism of *Periplaneta orientalis* concluded that a similar mechanism existed in the roach for the supply of energy, in the anaerobic phase of muscle contraction, as in higher animals, viz., a glycogen to lactic acid breakdown in the absence of oxygen. Boell (1935) without referring to glycogen *per se*, suggested that a low respiratory quotient, following an initial value of close to unity in the embryonic development of *Melanoplus differentialis*, meant a fat to carbohydrate conversion in the later stages. Hitchcock and Haub (1941), in a study on metabolism of the various

stages of the blowfly (*Phormia regina*) also assumed that, an R.Q. of 0.77 falling to 0.50 during metamorphosis, correlated with a rise in carbohydrate in the form of reducing sugar, meant a fat to carbohydrate conversion. Steinhart (1935) found at hatching of locust eggs almost twice as much adenosine triphosphate (ATP), a primary source of energy, as at the beginning of egg development. Heller (1936) also found a low ATP content during diapause of butterfly (*Deiliphila euphorbiae*) pupae, with a high content in the active pupa. Male adults, generally more active than females in this species, were found to have a higher ATP content. Heller concluded that glycolysis must necessarily be high with increased muscular activity, and that an ATP enzyme system was involved here simialr to that of higher animals. "ATP is the pivot around which the cycle of phosphoric exchanges of glycogenolysis revolves", suggested Heller. Baldwin and Needham (1934) had previously found ATP in the muscles of *Calliphora* and *Lucilia* spp. together with reducing sugar in the form of a hexose monophosphate. Since the latter increases with muscle stimulation (Cori, 1941), the findings of Baldwin and Needham are an important contribution to the correlation of muscular activity with glycolysis in insects. Thompson and Bodine (1938) also found a high ATP concentration at the beginning of egg development, which fell to a minimum during diapause in *M. differentialis* grasshopper eggs. This corresponded to the findings of Boell (cited above) of an R.Q. of one at the beginning of egg development falling during diapause. Gilmour (1941), in a study of aerobic and anaerobic metabolism in larvae of *Tenebrio molitor*, found ten times as much glycogen consumed in anaerobiosis with a corresponding higher lactic acid production than in aerobiosis. However, during recovery from anaerobic metabolism lactic acid disappeared but with no significant increase in glycogen content. Albaum and Kletzkin (1948) isolated ATP from *D. melanogaster* and stated that it was apparently "identical with that obtained from mammalian muscle." Albaum (1949) also found ATP in two species of Coleoptera, one species of Isoptera and one species of Orthoptera. The enzyme adenosine triphosphatase (ATP-ase) was isolated by Gilmour (1948) from grasshopper myosin, prepared from the thoracic and hind femoral muscles. Its enzyme activity was tested against both rabbit and grasshopper ATP, and it was found to split both labile phosphates of ATP.

In the past two years significant contributions to the knowledge

of glycolytic processes in insects have been made by Barron and Tahmisian (1948) and Humphrey (1949). The former workers, in enzymological studies of metabolism of insect muscles, by methods developed in the study of muscle metabolism of higher vertebrates, found that leg muscles of the adult roach (*P. americana*) showed similar chemical pathways to those found in mammalian muscle in aerobic metabolism. Certain discrepancies were found in insect anaerobic glycolysis, however, since iodacetic acid, which inhibited CO_2 production, had no effect on lactic acid formation. Addition of ATP resulted in the disappearance of the CO_2 inhibition by iodacetic acid. Humphrey, in his glycolysis studies, reported he could obtain both lactic and pyruvic acid from homogenized leg muscles of the same species of roach, as well as from grasshopper muscle (*Locusta migratoria*). However, he and Siggins (work to be published) reported a glycolytic pattern not typical of vertebrates, although acid production from all 6-carbon intermediates usually associated with vertebrate glycolytic reactions did occur, in the grasshopper.

IV. Summary

Glycogen metabolism of insects was reviewed from *three* main aspects; glycogen occurrence in insects; the physiological basis of metabolism of glycogen in insects; chemical aspects of insect glycogen metabolism.

It was shown that certain species of insects are capable of utilizing glycogen in their diets and that glycogen is stored primarily in the fat body, the latter described by some workers as having the same function as the liver of higher animals with respect to glycogen metabolism.

Extensive studies on the presence of glycogen in the various stages of development, and its association with other nutrients at these times, have shown that glycogen is a storage form, which is usually built up to a high level by the active feeding larvae of holometabolous insects. This storage reaches a maximum just before pupation, glycogen content falling during metamorphosis from pupa to adult as the glucose content rises to a maximum just before adult emergence. In insects where glycogen was absent, it was suggested that fat or protein conversion (to sugar) takes place to account for the presence of reducing sugars in conjunction with respiratory quotients reported in such species. In the case of certain flying species, the importance of glycogen as the limiting factor in the duration of flight has been demonstrated.

Finally some of the chemical aspects of glycogen metabolism were discussed. The presence of adenosine triphosphate (ATP) has been associated with a respiratory quotient indicative of carbohydrate metabolism during development, and with storage and utilization of glycogen generally. High ATP content has been associated with glycolysis experimentally, and low ATP with increased glycogen storage, to establish a fundamental basis for glycogen metabolism in insects. The isolation of adenosine triphosphatase and very recent enzymological studies suggest that the chemical processes involved in glycogen metabolism in insects follow a pattern not dissimilar from that already established in higher animals.

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